High-Resolution Intravascular Magnetic Resonance Imaging Monitoring of Plaque Formation in Heritable Hyperlipidemic Rabbits

Gesine G. Zimmermann-Paul, MD; Harald H. Quick, MS; Peter Vogt, MD; Gustav K. von Schulthess, MD, PhD; Dorothee Kling, PhD; Jörg F. Debatin, MD

Background—The individual makeup of atherosclerotic plaque has been identified as a dominant prognostic factor. With the use of an intravascular magnetic resonance (MR) catheter coil, we evaluated the effectiveness of high-resolution MR in the study of the development of atherosclerotic lesions in heritable hyperlipidemic rabbits.

Methods and Results—Sixteen hyperlipidemic rabbits were investigated at the ages of 6, 12, 24, and 36 months. The aorta was studied with digital subtraction angiography and high-resolution MR with the use of a surface coil and an intravascular coil that consisted of a single-loop copper wire integrated in a 5F balloon catheter. Images were correlated with histological sections regarding wall thickness, plaque area, and plaque components. Digital subtraction angiography revealed no abnormalities in the 6- and 12-month-old rabbits and only mild stenoses in the 24- and 36-month-old rabbits. High-resolution imaging with surface coils resulted in an in-plane resolution of $234 \times 468 \, \mu m^2$. Delineation of the vessel wall was not possible in younger rabbits and correlated only poorly with microscopic measurements in the 36-month-old rabbits. Intravascular images achieved an in-plane resolution of $117 \times 156 \, \mu m^2$. Increasing thickness of the aortic wall and plaque area was observed with increasing age. In the 24- and 36-month-old animals, calcification could be differentiated from fibrous and fatty tissue on the basis of the T2-fast spin echo images, as confirmed by histological correlation.

Conclusions—Atherosclerotic evolution of hyperlipidemic rabbits can be monitored with high-resolution intravascular MR imaging. Image quality is sufficient to determine wall thickness and plaque area and to differentiate plaque components. (Circulation. 1999;99:1054-1061.)

Key Words: atherosclerosis • magnetic resonance imaging • balloon • catheters • imaging

Atherosclerosis represents a chronic inflammatory response to injury ending in an acute event induced by plaque rupture.1,2 Originating from fatty streaks, the typical advanced lesion consists of a fibrous cap overlying a central necrotic core that contains cellular debris, abundant extracellular lipid, and foam cells.2,3 This particular plaque configuration carries a high risk of fissure and ensuing thrombosis independent of the degree of associated luminal narrowing.4 Thus, rather than the degree of stenosis, the risk for thrombosis appears to be determined by plaque structure.5 Hence the assessment of atherosclerosis must extend beyond mere angiographic depiction of the arterial lumen.

Driven by continuous improvements in magnetic resonance (MR) hardware and software designs, high-resolution MR imaging (MRI) has been increasingly considered for assessing the vascular walls. With the use of specialized coils that permitted scanning of only ex vivo specimens, high-resolution MR images were found to be superior to intravascular ultrasound with regard to plaque characterization, reflecting the unsurpassed soft tissue contrast inherent to the MR experiment.6 Initial in vivo work was based on external surface coils. Limited signal and depth penetration allowed wall imaging only of peripheral vessels such as the carotid,7 femoral, or popliteal arteries. To overcome these limitations, various intravascular imaging coils have been designed.8–11 Recently, a design based on a single-loop receiver coil mounted on an inflatable balloon catheter has been introduced.12 The device was shown to render sufficiently high and homogeneous signal to resolve ex vivo plaque13 while suppressing flow artifacts under in vivo conditions.

The purpose of this study was to monitor the age-dependent development of atherosclerotic lesions in heritable hyperlipidemic rabbits by use of a high-resolution intravascular imaging catheter and compare its performance with that of x-ray angiography and high-resolution MR with an external surface coil, with histological analysis used as the standard of reference.

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tracheal tube (Portex Ltd). Halothane narcosis was subsequently performed under spontaneous breathing conditions with 1.5% halothane and a 60%/40% O2/N2O mixture. For DSA, a 4F introducer was placed from the right carotid artery into the aortic arch. For placement of the intravascular MRI catheter, the right femoral artery was surgically prepared and sectioned. After the animals were killed by injection with pentobarbitol (Veterinaria AG), histological sections of the abdominal aorta were obtained.

Digital Subtraction Angiography

DSA images of the abdominal aorta were acquired with a frame rate of 2 per second after administration of 5 mL of Topromid (Schering AG) with the use of standard angiographic equipment (Multiskop Siemens). Separate series of the suprarenal and infrarenal abdominal aorta were obtained with a 1024×1024 matrix.

Magnetic Resonance Imaging

All MRI was performed on a 1.5-T system (Signa Echospeed, GE Medical Systems). The rabbit was placed in a quadrature extremity coil centered on the abdomen of the animal. With the use of a series of fast gradient echo localizing sequences, a 40-mm region of the suprarenal aorta was defined for high-resolution MRI with both the external and intravascular coil approaches. To permit direct comparison, 4-mm sections were obtained with both techniques in the axial plane.

For noninvasive high-resolution imaging, the extremity surface coil was used for both signal transmission and reception. T1-weighted spin-echo images (TR/TE=500/12 ms, 4NEX) were acquired in 8.36 minutes. A fast spin-echo sequence (TR/TE=2000/85 ms, Etl=8, 8NEX) rendered T2-weighted images also in 8.36 minutes. To reduce flow-related artifacts, both superior and inferior spatial presaturation pulses were used. With each sequence, 10 contiguous 4-mm sections were collected with a field-of-view of 120×120 mm and a 512×256 matrix.

For intravascular MRI, the balloon-mounted intravascular coil was used for signal reception. The design of the intravascular catheters (Schneider International) was based on a standard 5F balloon catheter with an inflatable balloon (4-mm diameters) of 40 mm in length. A single-loop coil, 40 mm in length and made of copper wire, was mounted onto the surface of the balloon (Figure 1).

To isolate the nonbiocompatible copper from the vessel, the wire was covered by a second balloon. A coaxial cable was used to conduct the MR signal over the length of the catheter (120 cm) and to connect the coil to remote tuning and matching capacitors \( C_t \) and \( C_m \) positioned at the base of the catheter. An actively switched diode \( D \) detunes the catheter receiving coil during transmission with the body coil (Figure 1). A sensitivity profile of the intravascular coil, acquired in a phantom, demonstrated a rather circular signal homogeneity and penetration depth (Figure 2).

The intravascular imaging catheter was placed at the predefined 40-mm-long region of the suprarenal aorta. T1-weighted spin-echo images (TR/TE=400/20 ms, 6NEX) were acquired over 3.54 minutes; T2-weighted fast spin-echo images (TR/TE=2000/85 ms, Etl=8, 8NEX) were collected in 3.16 minutes. With both sequences, 10 contiguous 4-mm sections were collected with a rectangular field-of-view of 30×15 mm and a matrix of 256×96.

Histopathological Evaluation

To ensure identification of the predefined supra-aortic region of interest, the intravascular catheter remained inside the aorta after the animals were killed. The aortic segment containing the balloon-mounted coil was carefully excised from the cadaver. To facilitate the matching process between MR images and histological specimen, the aorta was marked with color (Viomedex, Hausmann Hospital Supply Ltd) at the distal and proximal ends of the inflated balloon as well as along the anterior vessel wall. After excision, the specimens were immediately fixed in 10% buffered formalin for at least 24 hours. For cutting, the specimens were oriented in the anterior-posterior plane and embedded in paraffin. Three sections, 4 μm in thickness, corresponding to the center of the 3 central MR.
images, were mounted on slides. For subsequent histopathological evaluation, the slides were stained with hematoxylin-eosin and elastic van Gieson and analyzed for fibrous and fat components. To confirm the presence of calcification or chondroid metaplasia, Kossa stain and AB PAS stain were obtained, respectively.

Data Analysis
Analysis of the MR images and the histological specimens was performed by separate observers blinded to the other findings. Maximum wall thickness and plaque area were measured in each of the 3 histological sections as well as the corresponding MR images. In cases of concentric wall thickening, the average of 3 measurements in 1 section was calculated. Plaque thickness and area were measured on MR images by tracing plaque contours manually with a magnification factor of 3. Histomorphometric measurements of wall thickness and plaque area were performed on a planimeter (MOP-OM3, Kontron). Because the internal elastic membrane of older rabbits showed fragmentation with focal media degeneration, the plaque area was histologically defined as the area between the lumen of the vessel and the external elastic membrane. On MR images, the plaque area corresponded to the region bordered by vascular lumen and adventitia.

Histopathological plaque analysis was provided by an experienced pathologist viewing the histological cross sections along the site of maximal wall thickness. Each analyzed pixel location was characterized as corresponding to 1 of the following plaque components: calcification, chondroid metaplasia, fibrous tissues, and fatty tissues. Rather than focal areas of homogeneous fat, atherosclerotic plaque in HHL rabbits contain varying amounts of crystallized cholesterol and foam cells mixed with fibrous tissue. Plaque contained within each section was characterized in accordance with the American Heart Association classification (type 1 to 6).

Analysis of the plaque structure on MR-images was accomplished by plotting the signal intensities along a straight line traversing the

Figure 3. DSA of abdominal aorta in 12-month-old rabbit does not reveal vascular abnormalities (A). Irregularities with some degree of stenosis (arrow) are apparent in a 36-month-old animal (B).

Figure 4. Spatial resolution inherent to high-resolution T2-weighted image (234×468 μm) acquired with surface coil is not sufficient to permit delineation of the vessel wall from the lumen in a 12-month-old rabbit (A). Thickening of the vascular wall (arrow) is barely seen in the 36-month-old rabbit (B).
plaque at its maximum size. To ensure homogeneous measurements, the intravascular coil had been positioned to encompass the wall measurement site within the 60 degree sector of the sensitivity field of the coil (Figure 2). To compensate for the effects of motion on plaque formation, the line plot traversed the plaque in a sagittal plane. By use of an analogous line, the signal intensities (SI) along the plaque seen on the MR images were recorded for every other pixel. The different pixel SI values could thus be directly attributed to the various plaque components seen at histological analysis. Mean SI values and standard deviations were determined for the various plaque components in any age group on either T1- or T2-weighted images.

mean standard deviation


definition of plaque components in any age group on either T1- or T2-weighted images.

Figure 5. High-resolution (117×156 μm) intravascular T2-weighted images with histopathological correlations (hematoxylin-eosin stain; original magnification ×25) transecting the abdominal aorta of 4 different animals aged 6 (A, B), 12 (C, D), 24 (E, F), and 36 months (G, H) in identical locations (white bar=5 mm). Wall thickness and plaque area increase with increasing age. Calcified plaque characterized by reduced SI on MR images (arrow in G) was proven in the histological hematoxylin-eosin stain (arrow in H). Fibrous tissue containing <50% fat was easily differentiated from calcification on the basis of higher SIs (arrowheads in G and H). The 0.035-in guide wire lumen is visible inside the inflated balloon (arrow).

Results

In rabbits aged 6 (n=4) and 12 months (n=4) and in 2 animals aged 24 months, DSA failed to demonstrate any vascular abnormalities in the abdominal aorta (Figure 3A). The remaining 6 animals (two 24 months old and four 36 months old) revealed contour irregularities with some degree of stenosis in the suprarenal and infrarenal parts of the abdominal aorta (Figure 3B).

High-resolution imaging with surface coils resulted in an in-plane resolution of 234×468 μm. This resolution was not sufficient to permit definitive delineation of vessel wall from vessel lumen and surrounding structures in the animals aged 6, 12, and 24 months. Thickening of the vascular wall was identified in the four 36-month-old rabbits (Figure 4). Correlation of wall thickness and plaque area based on these 4 animals was poor, as reflected by correlation coefficients of $r=0.71$ and $r=0.66$ for thickness and area, respectively. The large pixel size did not permit characterization of plaque components in any age group on either T1- or T2-weighted images.

With intravascular high-resolution images, an in-plane resolution of 117×156 μm was achieved. The vascular wall was easily delineated in all 16 animals of all age groups (Figure 5). Wall thickness and plaque area correlated well with the morphometric measurements of the histological specimens, as evidenced by the high correlation coefficients of $r=0.96$ and $r=0.98$ for thickness and area, respectively (Figure 6). There was, however, some systematic overestimation by MRI relative to the true values.

On the basis of analysis of the intravascular images, the progression of atherosclerotic changes over time was clearly visible (Figure 5). With increasing age of the animals, the aortic wall thickened from 0.56±0.07 mm for the 6-month-old rabbits to 2.41±0.35 mm for the 36-month-old rabbits. Similarly, plaque area increased from 6.75±1.24 mm$^2$ at 6 months old to 23.75±3.86 mm$^2$ in corresponding age groups (Table 1). Differences in plaque area between 6 and 12 months failed to be statistically significant ($P>0.05$). All other wall thickness and plaque area comparisons between the 4 different age groups fulfilled the criteria for statistical significance ($P<0.05$).

Table 2 summarizes the histological plaque characterization according to the AHA classification; Table 3 depicts the grading on the basis of the MR classification. Table 4 reveals good correlation between the AHA and MR classifications, with an overall agreement of 87.5%.

Reflecting limited soft tissue contrast, T1-weighted images did not permit differentiation of plaque components in any of the animals regardless of age. T2-weighted fast spin echo images, on the other hand, did allow definitive differentiation of calcified plaque characterized on the basis of SI values. Calcification (129.3±29.9) could be differentiated from fatty

evidence of fat or calcium; MR grade II: wall thickening with lipid deposits without calcium; MR grade III: wall thickening with evidence of fat and calcium.

The MR gradings were correlated with the histological AHA classification. For this purpose, the AHA classification was simplified by lumping together types I and II, types III and IV, and types V and VI lesions.

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tissue (595.2±146.8) and fibrous tissue (932.4±127.7). Differences between the 3 components were statistically significant ($P<0.01$) (Table 5) (Figure 7). The MRI appearance of chondroid metaplasia with SI values of 137.5±31.4 was indistinguishable from that of calcified plaque.

**Discussion**

This study illustrates that intravascular MRI is feasible for detecting early atherosclerosis and characterizing more advanced plaque formations. In contrast to the externally placed surface coil, the intravascular imaging device provided sufficient spatial resolution to accurately quantify wall thickness as well as plaque area and differentiate various plaque components on the basis of their inherent SIs (Figure 5). The presented data also underscore the limitations inherent to luminal angiographic imaging. DSA failed to identify plaque formation in younger animals altogether and grossly underestimated the true extent of disease in the older animals (Figure 3). Similar observa-

**TABLE 1. Development of Wall Thickness and Plaque Area on MR**

<table>
<thead>
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<th>Wall Thickness, mm</th>
<th>Plaque Area, mm$^2$</th>
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<tbody>
<tr>
<td>6 mo</td>
<td>0.56±0.07</td>
<td>6.75±0.95</td>
</tr>
<tr>
<td>1 y</td>
<td>0.98±0.18</td>
<td>8.75±2.21</td>
</tr>
<tr>
<td>2 y</td>
<td>1.72±0.33</td>
<td>14.25±2.98</td>
</tr>
<tr>
<td>3 y</td>
<td>2.41±0.35</td>
<td>23.75±3.86</td>
</tr>
</tbody>
</table>

All measurements are mean±SD.
tions have been reported by Hong et al\textsuperscript{18}: Intravascular ultrasound had identified circumferential wall thickening in 6-month-old heterozygous HHL rabbits, whereas angiography failed to detect any changes.

MR-based visualization of the vascular wall requires adequate spatial resolution coupled with sufficient signal strength and homogeneity. By providing direct contact between the imaging coil and the vessel wall, the evaluated balloon-mounted coil design ensures maximal spatial resolution. Positioning the coil next to the vascular wall allows the latter to fall within the area of maximal coil sensitivity. Hence, sufficient signal is received to provide adequate spatial resolution coupled with sufficient signal strength.

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Table 2. Number of Animals Showing Atherosclerotic Lesions on Histology According to AHA Classification of Atherosclerosis

<table>
<thead>
<tr>
<th>AHA Classification</th>
<th>I+II</th>
<th>III+IV</th>
<th>V+VI</th>
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<tbody>
<tr>
<td>6 mo</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 y</td>
<td>2</td>
<td>2</td>
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<td>2 y</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3 y</td>
<td>0</td>
<td>0</td>
<td>4</td>
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Table 4. Correlation of MR Gradings With Histological AHA Classification

<table>
<thead>
<tr>
<th>MR Type</th>
<th>AHA</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>I+II</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III+IV</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vb+VII</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
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</table>

Overall agreement was 87.5%.

MRI of vascular walls can also be accomplished with external surface coils.\textsuperscript{26} The relatively poor results obtained in this study primarily reflect differences in the animal model: Whereas Skinner et al\textsuperscript{26} used a deendotheliazation model resulting in well-developed atherosclerotic plaque, this study included rabbits with very early-stage disease. On the basis of an in-plane resolution of $343 \times 468 \, \mu m$, a mean wall thickness of 2.4 mm was required to permit definitive delineation of the aortic wall.

Several characteristics supported the choice of the HHL rabbit as a model for this study. The documented similarity between rabbit and human atherosclerotic plaque formation was featured most prominently.\textsuperscript{15,16} In addition, the quick development of the disease over months, coupled with a robustness of the animals ensuring survival over a period of 3 years,\textsuperscript{16} allowed the study of disease progression over time.

On the basis of its excellent soft tissue contrast, MRI even provides a means for characterizing plaque structure.\textsuperscript{7,13,27,28} Despite the greater heterogeneity characterizing plaque in the animal model that was used, T2-weighted images permitted differentiation of fibrous tissue from lipid deposits (Table 5 and Figures 5 through 7). The lower SI of the fatty material reflects the shorter T2 relaxation times of fat compared with fibrous material.\textsuperscript{7} The ability to differentiate the various plaque components reflects on the excellent soft tissue contrast inherent to the MR experiment. Intravascular MR images even permitted classification of plaque. The outlined MR grading scheme correlates well with a simplified AHA classification (Tables 2, 3, and 4). There was some underestimation of disease extent in 2 of the 12-month-old rabbits. This appears to reflect difficulties in visualizing lipid deposits in the preatheromatous stage on MR images. The overall good correlation points to a considerable potential of intravascular MRI regarding the characterization of plaque.\textsuperscript{29} The wide standard deviation of SIs associated with the various plaque components does point to limitations of the intravascular imaging concept as proposed: There is a considerable signal dropoff from the center of the coil. These difficulties
can be overcome by implementing rather complex correction algorithms, as proposed by Atalar et al.29 With these algorithms, the correlation can be expected to be even greater.

The concept of intravascular MRI must be viewed as part of a larger effort exploring the potential of using MRI for guiding and monitoring intravascular interventions. Driven by profound hardware and software innovations, including the development of open-configuration MR scanners that provide direct access to the patient during imaging,30 and ultrafast 3-dimensional MRI permitting a detailed display of entire vascular territories, 31,32 much progress has been made in this regard. With the use of different active tracking algorithms, intravascular guide wires and catheters can be visualized relative to surround-

ing structures in real time simultaneously in multiple planes.33,34 The first MR-guided vascular interventions were recently performed under in vivo conditions.35 By accurately quantitating plaque extent and characterizing plaque structure, intravascular MRI will be an important addition to the concept of interventional MR angiography. It promises to provide a prognostic data link regarding the outcome of the most common intravascular procedure: percutaneous transluminal angioplasty.

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References


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